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Changes in Blood Carbohydrate and Protein Titers During Morphogenesis of Silkworms. Masaji S. Nishimura and Ayako Ohsawa (Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553 Japan) Received August 1, 1975. *Botyu-Kagaku* 41, 15, 1976.

4. カイコの形態形成にともなう血中炭水化物とタンパク質の変動

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カイコでは，血中トレハロースは雌雄とも5令および蛹の前半期に増加し，蛹化および羽化前に減少した．一方，去勢雌では成虫化前に正常個体のような減少はみられず，これより同時期でのトレハロースは卵形成にある程度使用されると思われる．去勢雄のトレハロースは正常個体のそれと変化なかった．

カイコの雌の血中タンパク質はトレハロースの場合と同様の変動を示した．一方，去勢雌では蛹化後期にも高い値がつづき，タンパク質もトレハロースと同様に，雌の卵形成に消費されるものと推定される．雄のタンパク質は熟蚕期から蛹化直前にかけて高い値を保っていた．雌雄間でタンパク質の量的な性差はみられなかった．

血中グルコースは雌雄共に殆んど検出されなかった．

エリサンの血中トレハロースは雌雄共量的に同じ変動を示した．ただエリサンでは蛹化時に別に一つのピークがみられた．

エリサンの血中タンパク質は，5令の初期から増加しはじめ，蛹化時に一旦減少し，以後再び増加して成虫化直前まで高い値のままとどまった．その間，性差はみられなかった．

除脳休眠蛹の場合，除脳後180日を経た個体でさえ，血中炭水化物，タンパク質共に蛹化時の値と変化なかった．

Introduction

Since Wyatt and Kalf demonstrated that the major blood sugar in insects is α-trehalose[1,2], a non-reducing dimer of α-glucose, many reports have appeared on the physiological role of trehalose as the circulating form of carbohydrate cellular food and as a biochemical characteristic substance of insects[3,4,5].

In holometaborous insects, the concentration of blood trehalose changes during normal physiological process. The protein concentration in

hemolymph also shows a similar change during molting and metamorphosis. Although the profiles of protein from insect blood and tissues suggest that the most abundant proteins, which mainly exhibit enzymatic properties[6,7], are not essentially different between larva and adult, there are well-marked quantitative and perhaps minor qualititative differences in the blood proteins of both sexes in various insects[8].

As there were little consistent data on blood trehalose and protein titers of both sexes from the larval to adult stages, we first determined the amount of trehalose and protein of male and female hemolymphs separately from the early 5th instar to adult emergence of both silkworms and Eri-silkworms. Secondly, the blood after castration was used for determination of sugars and protein titers in order to confirm the effect of sex difference. Thirdly, the brainless diapausing pupae were used for comparison of the amount of blood sugar and protein after the arrest of metamorphosis.

The present paper deals with the changes in blood sugars and protein titers in normal and castrated males and females during metamorphosis and in debrained diapausing pupae.

Materials and Methods

Silkworm, *Bombyx mori*, and Eri-silkworm, *Samia cynthia ricini*, were used as the source of blood. The former was reared on fresh mulberry leaves during the whole larval life and the latter on artificial diet. Males and females were separately reared from the 4th instar stage onward.

Blood was collected by cutting legs or skin into an ice-cooled tube containing phenylthiourea every other day from the 1st day of 5th instar to 1-2 days after emergence, and stored frozen until assayed. A quadruple volume of saline solution was added to the blood immediately after thawing (final 5× diluted blood) and centrifuged at 2500×g for 2 minutes to remove blood cells and other tissue fragments. The resulting supernatant was used for assays of sugars and protein.

In castration experiments, both gonads were removed from silkworm larva on the 3rd day of the 4th instar larva without anesthesia and the wound was left intact after the blood had been wiped off. Corresponding wounds without removing the organs were made on the controls.

For experiments on the arrest of metamorphose, the brain of Eri-silkworm was removed within 4-20 hours after pupation under anesthesia by chilling and the wound was coated with melted paraffin. All pupae thus operated entered the diapause permanently. These brainless diapausing pupae served as sources of blood 3-180 days after operation.

Reduced sugars were determined by Scott and Melvin's method[9], trehalose by Wyatt's method[2] and protein by micro-biuret method using bovine serum albumin as a standard[10]. Glucose was eventually estimated as the remainder after the amount of trehalose was subtracted from the total reduced sugar amount.

Results and Discussion

Silkworm—trehalose

In the case of female silkworms, the blood trehalose titer reached maximum level at the 3rd day of the 5th instar, and thereafter decreased slowly to minimum level on the 3rd day of the

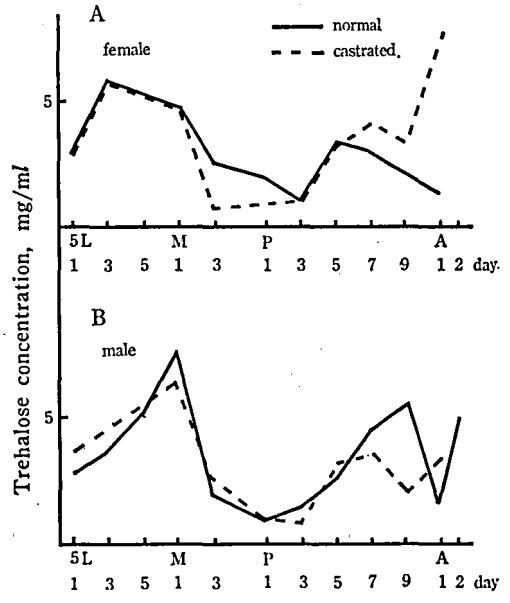

Fig. 1. Changes in blood trehalose titer during morphogenesis of silkworm.
L: larval stage; M: mature larval stage; P: pupal stage; A: adult stage.

pupal stage. It again increased at the middle of the pupal stage, then decreased during imaginal differentiation (Fig. 1A). On the other hand, in the case of males, the titer of blood trehalose reached maximum level in the mature larval stage, and the minimum level in the early pupal stage, in females. Then it increased again and reached the second peak at the 9th day of the pupal stage and suddenly decreased at the time of emergence (Fig. 1B). Generally, the blood trehalose in both sexes increased after maturation to pupation and increased again in the post-intermolting stage.

The trehalose increase during the first half stages of the 5th instar larva and pupa seemed to depend on the inhibition of trehalase activity in the hemolymph during the intermolts[11].

According to Candy and Kilby[12,13], the hemolymph trehalose is used not only for metabolic purposes, but also for providing carbohydrate material during chitin synthesis by the epidermis cells which appear to lack trehalase[14,15]. Epidermis cells utilize glucose liberated by the enzymatic hydrolysis of blood trehalose. Therefore, the fact that the trehalose amount decreased at the latter half of the 5th instar and the pharate adult stages may result from consumption for the new cuticle formation.

The time when the amount of blood trehalose was decreasing at the later pupal stage corresponded to the stage of egg maturation and/or yolk deposition. Since the blood trehalose of pupae gonadectomized in the 4th instar larval stage increased during the late pupal stage rather than decreasing, some trehalose may also be consumed during the process of egg development. On the other hand, the concentration of blood trehalose of castrated males was at a slightly lower level than the normal ones at the same stage.

Silkworm—glucose

Generally, trehalose is hydrolyzed by trehalase to glucose which is utilized for metabolism of the cells. Blood glucose was scarcely detected during the lst to 5th days of the 5th instar larva when the trehalose level was very high but reached the maximum level at about the 3rd day of the

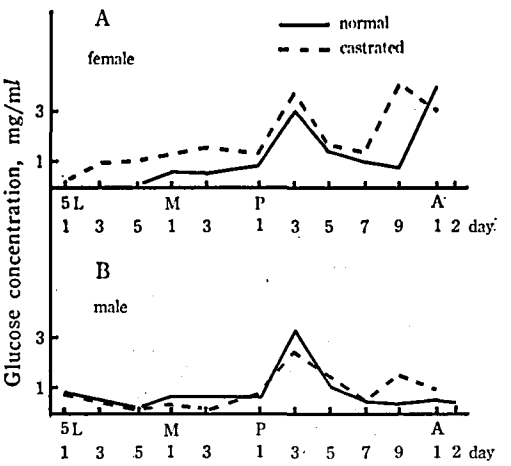

Fig. 2. Changes in blood glucose titer during morphogenesis of silkworm. Symbols are explained in Fig. 1.

pupal stage when the fall of blood trehalose was observed. The periods of increasing glucose corresponded to the intermolt stage (Fig. 2).

In the case of castrated females (Fig. 2A), as glucose was consumed only by imaginal differentiation but not by egg formation, the increase of blood glucose during the later part of adult development seemed to be reasonable.

On the other hand, in the case of males (Fig. 2B), since the gonadal development and differentiation had gradually progressed from the 5th instar larval stage, castrated specimens showed a similar pattern to normal ones for both trehalose and glucose.

Silkworm—protein

In the case of females (Fig. 3A), the level of hemolymph proteins increased during the 5th instar larval stage and reached maximum in the mature larval stage. From the prepupal stage to pupation, the protein titer fell slowly and rose again at the 5th day after pupation, then it decreased suddenly just before adult emergence. The fat body is an important site of synthesis and storage of proteins and begins to histolyze in the early pupal stage (before and after the 3rd day of pupa) especially in females[16,17]. A great amount of protein granules liberated into the blood following histolysis of the fat body, would cause temporary increase of the blood

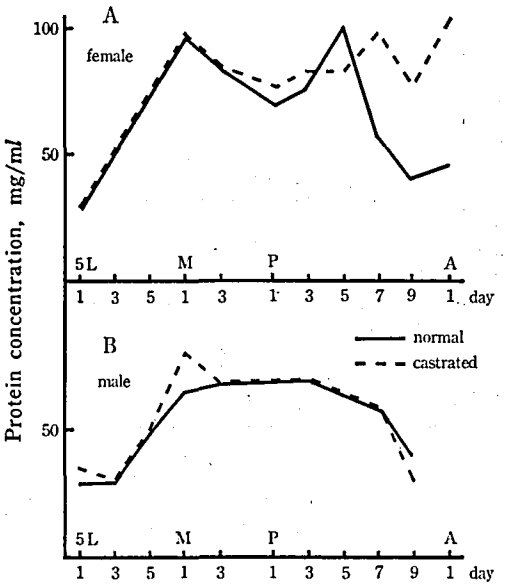

Fig. 3. Changes in blood protein titer during morphogenesis of silkworm. Symbols are explained in Fig. 1.

protein at the middle pupal period. The high concentration of blood protein thus accumulated seems to be also utilized for the female specific metabolism. The fact that in the castrated females, blood protein was kept in a rather high level from the 3rd day of the pupal stage to adult emergence, when compared with normal females, may support our conjecture.

In males (Fig. 3B), protein increased during the 1st day of the 5th instar to the mature larval stage as in females. Contrary to females, a constant level of blood protein titer continued after maturation to 2 days before emergence. It is not easy to explain why the different tendency of the protein titers in the sexes appeared after maturation. Since the blood protein titer of the castrated males did not differ from the normal specimens, it might depend on the difference of gonadal development. Spermatogenesis becomes more active at the end of the 5th instar larval stage[18] and after pupation, protein or sugars would not be much utilized for the development of male gonads when the egg develops in the female. This may be the reason that the protein level was constant during the pupal stage.

Eri-silkworm—trehalose, protein

Blood trehalose of both male and female Eri-silkworms showed the same change pattern at a similar level (Fig. 4). That is, it reached the maximum level from the middle stage of the 5th instar larva to the mounting stage, then decreased rapidly to the minimum level before pupation.

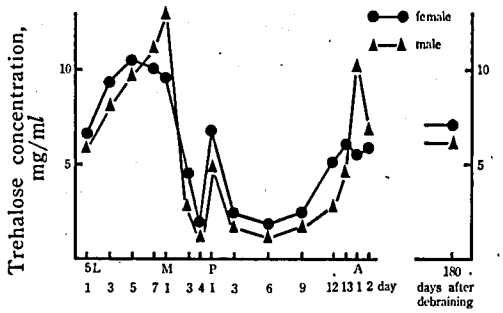

Fig. 4. Changes in blood trehalose titer during morphogenesis of Eri-silkworm. Symbols are explained in Fig. 1.

It increased again at pupation and quickly fell back to the minimum level again in the 3rd day of the pupal period. During the 3rd-9th days of the pupal stage, it remained at a low level and increased again during the late pupal to the adult stages. Except for the point of the second increase at pupation, the general tendency of blood trehalose in both *Bombyx* and *Samia* seemed to be the same.

Protein in blood of normal females and males increased from the early 5th larval stage, decreased a little at pupation but increased again

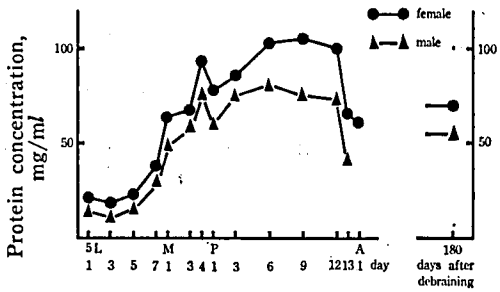

Fig. 5. Changes in blood protein titer during morphogenesis of Eri-silkworm. Symbols are explained in Fig. 1. Protein titer 180 days after debraining is shown on the right side.

during the middle pupal stage. It remained at a high level until just before adult emergence (Fig. 5).

The amount of protein in female blood was much high than that in males after maturation to the adult. This may be due to the fact that female specific protein[19] starts to appear from the late 5th instar larva and increases in number and amount during the pupal stage.

Protein in blood was also investigated using debrained pupae (Fig. 5, left end). Even in brainless pupae 180 days after operation, the protein titer was almost unchanged compared with normal pupae just after pupation, same as seen in the case of sugar.

These facts show the reliability of the arrest of morphogenesis, caused by the permanent artificial diapause upon removal of the brain.

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Summary

1. Changes in titer of the blood sugars and protein during morphogenesis of *Bombyx* and *Samia* were investigated.

2. Generally, in both females and males of *Bombyx*, blood trehalose increased during the first half stages of the 5th instar larva and pupa, and decreased before pupation and emergence.

3. In castrated females, the amount of trehalose did not decrease before emergence unlike in normal females. This suggests that some trehalose was consumed for egg formation and/or yolk deposition. In castrated males, the blood titer of trehalose was the same as in normal ones.

4. Change in titer of the blood protein of female *Bombyx* was similar to that of trehalose, although the two peaks had shifts of 2-3 days after the trehalose peaks. On the other hand, in castrated females, it remained at a high level during the late pupal stage. This suggests that some protein is utilized for egg formation, as with trehalose.

5. Contrary to females, male blood protein remained at a high level after maturation to just before pupation. No sex difference appeared in the protein titer between females and males.

6. Glucose, both in females and males, was scarcely detected in the blood except in the intermolt stages.

7. Blood trehalose of *Samia* in both females and males showed the same change with a similar level. The general tendency of both *Bombyx* and *Samia* seemed to be the same except that only *Samia* had an additional peak at pupation.

8. The blood protein level also showed no sex difference, protein increased in titer from the early 5th larval stage and decreased once at pupation, then remained at a high level until just before emergence.

9. In the case of debrained diapausing pupa, both blood sugars and protein did not change even after 180 days, showing exactly the same titer as normal pupa just after pupation.

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Effect of Tepa, Metepa and Hempa on the Bionomics of *Dacus cucurbitae* Coq. Serajuddin KHAN (Department of Zoology, Muslim University, Aligarh, India) Received August 4, 1975. *Botyu-Kagaku* 41, 20, 1976.

5. ウリミバエ *Dacus cucurbitae* Coq. の生態におよぼす tepa, metepa および hempa の影響

Serajuddin KHAN (Department of Zoology, Muslim University, Aligar, India) 50. 8. 4. 受理

ウリミバエ *Dacus cucurbitae* の雌雄を tepa, metepa, hempa で処理し，処理された個体と無処理，処理された個体間で交配し，その産卵数，孵化率，不妊率，産卵期間，成虫の生存期間を調べた．得られた結果は次のようである．1) 雄は雌より不妊剤に対する感受性が大きい．2) いずれの不妊剤も雌の産卵数を減少させたが，特に hempa はその作用が強く，0.5%で産卵が全くなかった．3) 産卵期間は不妊剤処理で短かくなった．4) 処理された成虫の寿命は雌では短かく，雄では長くなる傾向があり，これは不妊剤を実用化する場合には有利な条件になるだろう．

Benschoter and Paniagua[1] showed that the longevity of *Anastrepha ludens* was not effected when it was fed on a diet containing 2.0% biotin and similar results were obtained by Haniotakis and Galachtiou[2] who exposed *Dacus oleae* to residual films containing 0.620 to 1.240 ml/m^2 of metepa for 0.5, 1.3 or 5.0 minutes. However, these findings relate to fruit flies other than *D. cucurbitae* and the author is not aware of any such studies concerning *D. cucurbitae*. It was therefore, considered desirable to find out if tepa, metepa or hempa could effect the bionomics of this species.

Method and Materials

Freshly emerged flies were obtained from normal laboratory colonies and were divided in two groups, one of which was allowed to feed on sugar treated with different concentrations of tepa, metepa or hempa for three days while the other was maintained on normal fly food. After 72 hours, the flies living on the treated food were also provided normal diet. Virginity of the females was self insured as copulation invariably starts in this species after about 15 days of emergence. Sexing was done after 7 days and single pair reciprocal crosses were established between treated and untreated males and females as also between treated males and treated females by placing them in small cages, 3 × 3″ in size and constructed of wire frames covered over by mosquito netting. Five pairs of each type were studied to find out the rate of oviposition, fertility of eggs and the longevity of the adults.

Results

The results obtained are presented in tables 1 to 3. All the chemicals tested, reduced the fecundity of the females but such reduction was more pronounced when the flies were treated with different concentrations of hempa. The sterility effects of the chemosterilants on males and females were different but the females were less susceptible to the chemosterilants than the males. This substantiates the earlier observations of Keiser *et al.*[3] who found that the females of *D. dorsalis*, *D. cucurbitae* and *C. capitata* were less susceptible to sterility effects of apholate, tepa, metepa and tretamine. Males treated with 0.0078, 0.0156, 0.03125 and 0.0625% tepa and mated with untreated females induced 25.05, 45.4, 71.9 and 81.48% net sterility as compared to 18.51, 25.46, 65.88 and 72.54% net sterility obtained when treated females were confined with the normal males. A similar pattern was observed in the case of metepa, where a net sterility of